IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of	:	**Confirmation No. 8202**
Hashime KANAZAWA et al.	:	Attorney Docket No. 2005_0741A
Serial No. 10/533,806	:	Group Art Unit 1625
Filed May 5, 2005	:	Examiner Rita J. Desai
PYRAZOLONAPHTHYRIDINE DERIVATIVES	:	**Mail Stop: AMENDMENT**

DECLARATION UNDER 37 C.F.R. ' 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Kouki Ishitani, the undersigned, a citizen of Japan, residing at 1-8-16, Sakae-cho, Soka, Saitama, Japan, do hereby declare:

1. That I am a co-inventor of the above-identified application.
2. That I graduated from Tohoku Pharmaceutical University on March 20, 1989 with a degree in Ph. D.
3. That after working for Ihara Chemical Industry Co., Ltd. as a researcher for 3 years, I joined Grelan Pharmaceutical Co., Ltd. in 1992 and I have been in charge of planning and conducting pharmacological studies regarding pharmacological active agents under research and development stage in Grelan.
4. That my Relevant Publications are as follows.
   1) Auranofin inhibits calcium uptake into opsonized-zymosan-stimulated neutrophils obtained from rats. K. Ishitani, A. Matsuura and H. Honda, Inflamm. Res., 44, 482-485, 1995.
   2) Xanthine derivatives inhibit the increase of intracellular Ca^{2+} concentration induced by acetylcholine in nasal gland acinar cells of guinea pig. K. Ishitani, K. Ikeda, H. Sunose, D. Wu, H. Honda and T. Takasaka, Eur. Respir. J., 8, 2114-2119, 1995.

3) Intracellular Ca^{2+} response induced by acetylcholine in the submucosal nasal gland acinar cells in guinea pigs. K. Ikeda, M. Ishigaki, D. Wu, H. Sunose, M. Suzuki, K. Ishitani and T. Takasaka, Am. J. Physiol., 268, L361-368, 1995.
4) Effect of trimebutine malate on the contractile response of isolated ileum from diabetic rats. M. Uchida, T. Iwata, Y. Sugiyama, K. Ishitani, H. Honda and Y. Sakai, General Pharmacol., 25, 505-508, 1994.
5) Induction of platelet activating factor in mice by the intravenous administration of a neutral fraction of Bakers' yeast mannan. T. Mikami, K. Fukushi, K. Ishitani, M. Ishitani, S. Suzuki and M. Suzuki, Lipids, 26, 1404-1407, 1991.
6) Influence of transglutaminase on the function of mouse peritoneal macrophages. K. Ishitani and M. Suzuki, Microbiol. Immunol., 33, 59-68, 1989.
7) Influence of arachidonate metabolism on enhancement of intracellular transglutaminase activity in mouse peritoneal macrophages. K. Ishitani, S. Ogawa and M. Suzuki, J. Biochem., 104, 394-402, 1988.

5. That in order to show that the claimed compound has unexpectedly superior PDE IV inhibitory activity and therefore is not obvious over EP 0 526 840 or Suzuki et al. (US 5,281,610), I have performed under my direction and control the following experiments. The particulars and results of the experiments are set forth below.

EXPERIMENTAL SECTION

1. Object

In order to demonstrate the advantages of the claimed compounds, a comparison study was performed between a claimed compound (Example No. 9 on page 68 of the specification; hereinafter referred to as the "inventive compound") and US '610 Compound 1, which is a molecule derived by removal of methylene from the inventive compound, (3,5-diphenyl-1H-pyrazolo [4,3-c] [1-8] naphthyridin-4(5H)-one; hereinafter referred to as the "US '610 compound") for their PDE IV inhibitory efficacies according to assay methods disclosed in the present specification.

2. Method

The assays for PDE IV activity were conducted according to Nicholson et al. method (Br. J. Pharmacol., 97, 889 (1989)).

PDE IV isozymes as used herein were separated from U937 culture cells by using an anion exchange chromatography. Type IV PDE isozyme was admixed with ethylene glycol (EG) to adjust the final EG concentration to 30%, then stored at -20℃ and diluted when used. The enzymatic activity for PDE IV was measured using cAMP as a substrate.

[^{3}H]-cAMP (962 GBq/mmol; Amersham, 25 µl (100,000 cpm)) was added together with PDE IV isozyme (25µl) to an incubation buffer solution with the composition given below to adjust the total volume to 250µl. Each test compound was dissolved in DMSO to adjust the final concentration to 1% (2.5µl /tube).

Incubation buffer solution (pH7.5):

Tris-HCl (50mM), magnesium chloride (6mM), dithiothreitol (2.5mM), 5-nucleotidase (4 µg/ml), bovine serum albumin (0.23mg/ml), and cAMP (1 µM).

A mixture of the aforementioned test compound solution and the buffer solution was incubated at 30℃ for 20 minutes. The reaction was quenched by admixing with 1 ml of anion

exchange resin slurry (AG1-X8, 200-400 meshes, chloride form; Bio-Rad) to absorb unreacted substrates. After the reaction stopped, the mixture was centrifuged at 800 x g for 10 minutes, and the resulting supernatant was collected with vials in 250 µl aliquots. To each vial was added 5 ml of ACS-II (scintillator, Amersham). The radioactivity was measured with a liquid scintillator counter for [^{3}H]-adenosine and set as the PDE IV activity.

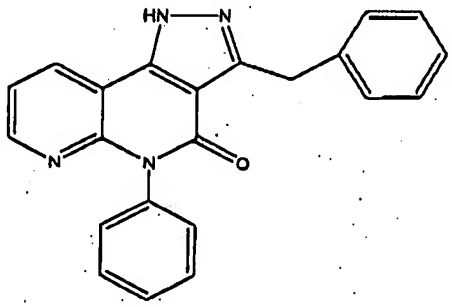
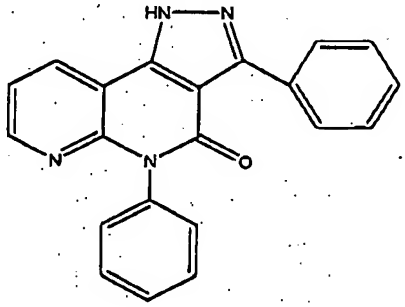
The % inhibition was calculated for the compounds, and IC_{50} (the concentration of each compound required for 50% inhibition) was obtained by Probit method.

The results are shown in Table 1 below.

3. Results

Table 1

PDE IV INHIBITION COMPARISON BETWEEN THE INVENTIVE COMPOUND AND THE US '610 COMPOUND

	The Inventive Compound	The US '610 Compound
Chemical Formula		
PDE IV Inhibition (IC_{50})	0.084 µM	0.25 µM

As apparent from Table 1, the PDE IV inhibitory efficacy (IC_{50} = 0.084μM) of the inventive compound is about 3 times higher than the PDE IV inhibitory efficacy (IC_{50} = 0.25μM) of the US '610 compound.

4. Discussion

Thus, the presence or absence of methylene greatly affects PDE IV inhibition activity. This study demonstrates the unexpectedly superior PDE IV inhibition activity of the inventive compound. This augmentation in PDE IV inhibition would not have been expected by a person skilled in the art.

5. Conclusion

I have carefully reviewed the file history and the rejections of record in this application, including the rejection of claims 1-24 under 35 U.S.C. § 103(a) as obvious over EP 0 526 840 or Suzuki et al. (US 5,281,610) in the Office Action dated May 31, 2007. Based on this review and analysis of the above-noted data, it is my professional opinion and belief that the invention of claims 1-24 is not rendered obvious by EP 0 526 840 or Suzuki et al. (US 5,281,610) as such references do not teach or suggest the unexpectedly superior PDE IV activity of the claimed compounds.

I further declare that all statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

Date: 12th Nov 2007

(Signature of Declarant)

Kouki Ishitani